Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 3, 5, 7, and 12 have been amended, and new claim 13 has been introduced. Descriptive support for the amendments to claims 3, 5, and 12, and for new claim 13 is provided in original claim 5 as well as page 7, lines 5-15, page 8, lines 1-8, and the Examples (*see* Example 8 re: adenocarcinoma). Therefore, no new matter has been introduced by these amendments.

Claims 3-9, 12, and 13 remain pending, with claims 3-5, 7-9, 12, and 13 under examination and claim 6 standing withdrawn.

This submission is accompanied by a request for a one-month extension of time. All fees should be charged to deposit account 14-1138. Any overpayment or underpayment should be applied to this same account.

Before addressing the rejections identified in the office action, applicants wish to explain once more the substance of the present invention. As stated in the description, the invention is based on the surprising finding that the growth and differentiation of non-HIV-infected tumor cells can be influenced by certain non-nucleoside reverse transcriptase inhibitors (NNRTIs), in particular those related to the nevirapine class of substances, efavirenz and delarvidine.

The inventors found that tumor cells possess endogenous, non-telomeric reverse transcriptase (RT) activity. RT activity is normally associated only with retroviral infections, such as infections with HIV. Although endogenous retroviruses have been shown to exist, it is believed that nobody had ever before shown that endogenous RT activity was linked to tumor development. Starting from this finding, the inventors demonstrated that certain NNRTIs are effective as anti-tumor drugs against a number of different tumor types due to their activity as RT inhibitors. As a consequence, the present inventors found a new use for the claimed class of NNRTI, namely the treatment of certain tumor pathologies which are associated with endogenous RT activity.

The rejection of claims 3-5, 7-9, and 12 under 35 U.S.C. § 112 (first paragraph) for lack of enablement is respectfully traversed.

At page 3 of the office action, the U.S. Patent and Trademark Office ("PTO") asserts that the present application does not provide enablement for any tumor or non-tumor pathology, but acknowledges that the application is enabling for a method of counteracting the loss of cellular differentiation and the treatment of particular tumor pathologies using efavirenz. Thus, there are two bases for the rejection: (i) the scope of tumor or non-tumor pathologies; and (ii) the scope of compounds encompassed by the claims.

In response to the first issue, claim 3 has been amended to recite a subgenus of tumor pathologies, specifically leukemia, epithelial tumors, tumors of the nervous system, fibro- and osteo-sarcomas. Claim 5 is further directed to several more precise types of epithelial tumors, while claims 12 and 13 are directed, respectively, to a particular tumor of the nervous system (glioma) and to a particular leukemia (AML). Thus, limiting the claim scope of tumor pathologies to be treated in accordance with the claimed invention – in consideration of the positive results in the Examples – effectively overcomes this first basis of rejection.

In response to the second issue, claim 3 has been amended to remove the objected language concerning "derivatives." In addition, as discussed more fully below, the specification explicitly recites that two members of the recited subgenus of NNRTI were demonstrated to be effective *in vitro* and/or *in vivo*. This effectively overcomes the second basis of rejection.

At pages 4-5 of the office action, the PTO asserts that experimental support is provided only for teratocarcinoma (F9), adenocarcinoma (HeLa) and osteosarcoma (Saos-2) cells in Examples 7-8. Applicants respectfully disagree, because a number of additional experiments are also described in the application and these additional sets of *in vitro* and *in vivo* data collectively support the breadth of tumor pathologies that can be treated in accordance with the claimed invention.

Figure 1 and Example 1 relate to the measurement of RT activity in various tumor cell lines, including leukemia (NB4, R4, Kasumi-1 and HL60 cells), teratocarcinoma (F9 cells), fibrosarcoma (L929 cells), osteosarcoma (Saos-2 cells), colon carcinoma (HT29 cells), breast carcinoma (MDA-231 and MCF7 cells), and glioma (U343 cells). As explained above, the invention is based on the findings by the inventors that all tested tumor cell lines possess endogenous, non-telomeric RT activity and that NNRTI have anti-tumor activity.

In addition, although there is no corresponding Figure, it is clearly indicated at page 7, lines 5-15, of the specification that the effects of nevirapine and efavirenz were tested on

cell lines already tested for RT activity in Figure 1, and that *both* compounds were efficient for decreasing the rate of cell proliferation and promoting cell differentiation. Thus, the efficiency of efavirenz and nevirapine for decreasing proliferation and promoting differentiation is demonstrated *in vitro* for leukemia (NB4, R4, Kasumi-1 and HL60 cells), teratocarcinoma (F9 cells), fibrosarcoma (L929 cells), osteosarcoma (Saos-2 cells), colon carcinoma (HT29 cells), breast carcinoma (MDA-231 and MCF7 cells), and glioma (U343 cells). This supports applicants' position that NNRTI as a class should have antitumor activity against all tumor types that show endogenous non-telomeric RT activity (such as those recited in claim 3).

Concerning hepatoma (another name of hepatocellular carcinoma or HCC), experiments *in vivo* with efavirenz are provided in Example 11 (*see* page 19) and Figure 12. The obtained results show that efavirenz efficiently decreases hepatoma tumor growth.

Thus, taking into account all of the experimental support identified in the present application, experimental evidence of the efficiency of both efavirenz and nevirapine species for decreasing tumor cell proliferation and promoting differentiation is provided for:

- leukemia, including four types of leukemia cells (NB4, R4, Kasumi-1 and HL60) (*see* page 7, lines 5-15);
- five types of carcinomas, which is synonymous with epithelial tumors (*see* attached Exhibit A – "carcinoma" definition from www.freedictionary.com): teratocarcinoma (F9 cells), colon carcinoma (HT29 cells), breast carcinoma (MDA-231 and MCF7 cells), adenocarcinoma (HeLa cells) (*see* page 7, lines 5-15, and Example 8), and hepatoma (*see* Example 11 and Figure 12);
- fibrosarcoma (L929 cells) (*see* page 7, lines 5-15);
- osteosarcoma (Saos-2 cells) (*see* page 7, lines 5-15, and Example 8); and
- a tumor of the nervous system; glioma (U343 cells) (*see* page 7, lines 5-15).

Given the extent of *in vitro* and *in vivo* data presented in the present application, claim 3 is clearly fully enabled for leukemia, carcinomas or epithelial tumors, fibrosarcoma, osteosarcoma, and tumors of the nervous system. In the same manner, claim 5 is fully enabled for all recited carcinomas, and claim 12 is fully enabled for glioma.

As additional support for the scope of the claimed invention, Applicants submit herewith a post-filing date publication by the inventors (Sciamanna et al., "Inhibition of Endogenous Reverse Transcriptase Antagonizes Human Tumor Growth," *Oncogene* 24:3923-

3931 (2005) ("Sciamanna") (attached as Exhibit B). Sciamanna confirms the results obtained with the five types of carcinomas, identified above, by reproducing these results using a sixth type of carcinoma, prostate carcinoma. In particular, Sciamanna shows that efavirenz efficiently reduces proliferation of a prostate carcinoma cell line PC3 *in vitro* (*see* Figure 1a) and *in vivo* (*see* Figure 6). Sciamanna demonstrates that efavirenz reduces the tumorigenic potential of PC3 prostate carcinoma cells *in vivo* (*see* Figure 7).

In addition, Sciamanna also demonstrates a beneficial effect of efavirenz for melanoma (*see* Figure 1a), a completely different type of cancer, thus demonstrating the wide range applicability of efavirenz for the treatment of cancer.

Because all tumor cell lines tested were found to express RT endogenous activity, regardless of tumor types, and the proliferation of all tested cell lines was inhibited by the tested NNRTI, namely efavirenz and nevirapine, applicants submit that one of skill in the art is fully able to practice the claimed invention for treatment of carcinomas (*i.e.*, epithelial tumors), leukemias, tumors of the nervous system, and fibro and osteo-sarcomas.

For all these reasons, the rejection of claims 3-5, 7-9, and 12 for lack of enablement is improper and should be withdrawn.

The rejection of claims 3-5, 7-9, and 12 under 35 U.S.C. § 112 (second paragraph) is respectfully traversed in view of the amendments to claims 3 and 7 to delete the objected-to language. This rejection should be withdrawn.

The rejection of claims 3-5, 7, and 12 under 35 U.S.C. § 102 (a) as anticipated by Murdaca et al., AIDS 16(2):304-5 (2002)("Murdaca") is respectfully traversed.

Murdaca discloses the case study of an AIDS patient with a typical AIDS related tumor, Kaposi's sarcoma ("KS"). After the onset of the disease, the patient was treated with three anti-HIV drugs simultaneously: two nucleoside reverse transcriptase inhibitors (zidovidine and lamivudine), and one non-nucleoside reverse transcriptase inhibitors (efavirenz).

The only tumor mentioned in Murdaca is KS. All sarcomas are mesenchymal tumors and not epithelial tumors (*i.e.*, carcinomas). KS is not a tumor of the nervous system, and it is distinct from leukemia, fibro- and osteo-sarcomas.

Claim 3 has been limited to the treatment of tumor pathologies selected from the group consisting of leukemia, epithelial tumors, tumors of the nervous system, fibro- and osteo-sarcomas. In view of these amendments, the claims do not read on the treatment of KS as taught

in Murdaca. Therefore, the rejection of claims 3-5, 7, and 12 as anticipated by Murdaca is improper and should be withdrawn.

The rejection of claims 8 and 9 under 35 U.S.C. § 103(a) for obviousness over Murdaca in view of U.S. Patent No. 6,235,733 to Bahal et al., ("Bahal") is respectfully traversed.

The teachings and deficiencies of Murdaca are noted above. As noted above, Murdaca discloses only the treatment of a patient suffering from AIDS and the related KS tumor. There is no mention of any other types of tumor. Bahal is cited at page 10 of the office action for teaching oral liquid formulations of efavirenz; however, the PTO has failed to demonstrate how Bahal overcomes the above-noted deficiencies of Murdaca. Therefore, the obviousness rejection of claims 8 and 9, which ultimately depend from claim 3, is improper and should be withdrawn.

As further evidence of non-obviousness, it must be considered what the prior art taught regarding NNRTI as therapeutics. In Murdaca, the patient was treated with three anti-HIV drugs simultaneously: zidovidine, lamivudine and efavirenz. Zidovidine and lamivudine are both nucleotide analogue RT inhibitors (referred to as NRTI or NARTI), whereas efavirenz is a non-nucleoside RT inhibitor (referred to as NNRTI). The patient was also simultaneously treated by trimethoprim-sulfamethoxazole and azithromycin, two antibiotic compounds. Thus, the only information that can be gathered from Murdaca is that a regression of KS was observed in association with a triple anti-HIV regimen and a double antibiotic treatment. In fact, there is absolutely no teaching of any effect of efavirenz or any other NNRTI on KS.

Since antibiotics were simultaneously administered in the reported case study, it is not even clear whether regression of KS was linked to the triple anti-HIV regimen. Murdaca even mentions in the discussion that liposomal daunorubicin and doxorubicin have been shown to be efficacious in the treatment of AIDS/KS without adverse effects on the CD4 count (*see* page 305, first full paragraph). Since daunorubicin and doxorubicin are anthracycline antibiotics used in cancer chemotherapy (*see* attached Exhibit C), this would suggest to the person of ordinary skill that antibiotics may be beneficial for KS treatment and, thus, the KS regression observed in Murdaca might be linked to the simultaneously administered antibiotics.

Moreover, even if it was not the case, since a triple anti-HIV regimen was used, it is impossible to differentiate between the effects of the three drugs which were given as a combination therapy. However, Murdaca discloses in its discussion that KS has been reported responding to a combination treatment comprising the same two NRTI (zidovidine and

lamivudine) and one protease inhibitor instead of efavirenz (*see* page 305, first full paragraph). This would have suggested to the person of ordinary skill that if the effect observed in Murdaca is linked to the triple anti-HIV regimen, then it may be attributed to the two nucleoside analog RT inhibitors (zidovidine and lamivudine) rather than to the efavirenz.

Finally, because KS is known as an AIDS-related disease that mainly develops in immune-defective patients, a skilled person would have considered it probable, as of the priority filing date, that KS regression may be simply linked to the observed, concomitant reduction of viral load (*see* increase in CD4 count and decrease of viral load, paragraph bridging pp. 304-305) and, therefore, associated with immune function improvement.

Therefore, at the priority date of the present application and based on the Murdaca disclosure considered as a whole, a skilled artisan would have attributed the effect on KS described in Murdaca to immune function improvement or activity of agents other than efavirenz. There is absolutely no suggestion that efavirenz or any other NNRTI would be expected to be useful to treat the tumor pathologies as presently claimed. Further, the PTO has failed to demonstrate where the prior art suggests that the recited tumor pathologies were associated with endogenous non-telomeric RT activity or that treatment of the recited tumor pathologies would have been expected to benefit from using the NNRTI as presently claimed.

For all these reasons, the rejection of claim 8 and 9 for obviousness over the combination of Murdaca and Bahal is improper and should be withdrawn.

In view of the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: October 22, 2009

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Exhibit A: Carcinoma definition from www.freedictionary.com

thefreedictionary.com

car·ci·no·ma (kär'sə-nō'mə)
n. pl. **car·ci·no·mas** or **car·ci·no·ma·ta** (-mə-tə)
An invasive malignant tumor derived from epithelial tissue that tends to metastasize to other areas of the body.

[Latin, *cancerous ulcer*, from Greek karkinōma, from karkinos, *cancer*, see kar- in Indo-European roots.]

car'ci·no'ma·toid (-nō'mə-toid') *adj.*

car'ci·nom'a·tous (-nōm'ə-təs, -nō'mə-) *adj.*

carcinoma [□k□□s□□nə□mə]
n pl **-mas**, **-mata** [-mətə] *Pathol*
1. (Medicine / Pathology) any malignant tumour derived from epithelial tissue
2. (Medicine / Pathology) another name for cancer [1]

[from Latin, from Greek *karkinōma*, from *karkinos* CANCER]

carcinomatoid , **carcinomatous** *adj*

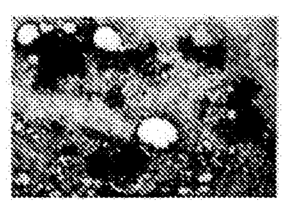
carcinoma (kär'sə-nō'mə)
Plural **carcinomas** or **carcinomata** (kär'sə-nō'mə-tə)
Any of various cancerous tumors that are derived from epithelial tissue of the skin, blood vessels, or other organs and that tend to metastasize to other parts of the body. See also basal cell carcinomasquamous cell carcinoma

carcinoma
1. a malignant tumor that may spread to surrounding tissue and distant areas of the body.
2. any kind of epithelial cancer. — **carcinomatous,** *adj.*

See also: Cancer

Thesaurus **Legend:** Synonyms Related Words Antonyms

Noun 1. **carcinoma** - any malignant tumor derived from epithelial tissue; one of the four major types of cancer



cancer of the liver, liver cancer - malignant neoplastic disease of the liver usually occurring as a metastasis from another cancer; symptoms include loss of appetite and weakness and bloating and jaundice and upper abdominal discomfort

cancer, malignant neoplastic disease - any malignant growth or tumor caused by abnormal and uncontrolled cell division; it may spread to other parts of the body through the lymphatic system or the blood stream

adenocarcinoma, glandular cancer, glandular carcinoma - malignant tumor originating in glandular epithelium

breast cancer - cancer of the breast; one of the most common malignancies in women in the US
carcinoma in situ, preinvasive cancer - a cluster of malignant cells that has not yet invaded the deeper epithelial tissue or spread to other parts of the body
colon cancer - a malignant tumor of the colon; early symptom is bloody stools
embryonal carcinoma - malignant neoplasm of the testis
endometrial cancer, endometrial carcinoma - cancer of the uterine lining
hepatocarcinoma, hepatocellular carcinoma, hepatoma, malignant hepatoma - carcinoma of the liver
lung cancer - carcinoma of the lungs; one of the commonest forms of cancer
mesothelioma - a form of carcinoma of the mesothelium lining lungs or abdomen or heart; usually associated with exposure to asbestos dust
oat cell carcinoma, small cell carcinoma - highly malignant carcinoma composed of small round or egg-shaped cells with little cytoplasm; lung cancers are frequently oat cell carcinomas
oral cancer - malignant neoplasm of the lips of mouth; most common in men over the age of 60
pancreatic cancer - cancer of the pancreas
seminoma, testicular cancer - malignant tumor of the testis; usually occurring in older men
skin cancer - a malignant neoplasm of the skin
trophoblastic cancer - malignant neoplasm of the uterus derived from the epithelium of the chorion

Translations

Select a language: -----------------------

carcinoma [ka□si'nəumə]

malignant tumor *The surgeons had to remove the carcinoma from his lungs*

Exhibit B: Sciamanna et al., "Inhibition of Endogenous Reverse Transcriptase Antagonizes Human Tumor Growth," *Oncogene* 24:3923-3931 (2005).

Oncogene (2005) 24, 3923–3931

www.nature.com/onc

Inhibition of endogenous reverse transcriptase antagonizes human tumor growth

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Undifferentiated cells and embryos express high levels of endogenous non-telomerase reverse transcriptase (RT) of retroposon/retroviral origin. We previously found that RT inhibitors modulate cell growth and differentiation in several cell lines. We have now sought to establish whether high levels of RT activity are directly linked to cell transformation. To address this possibility, we have employed two different approaches to inhibit RT activity in melanoma and prostate carcinoma cell lines: pharmacological inhibition by two characterized RT inhibitors, nevirapine and efavirenz, and downregulation of expression of RT-encoding LINE-1 elements by RNA interference (RNAi). Both treatments reduced proliferation, induced morphological differentiation and reprogrammed gene expression. These features are reversible upon discontinuation of the anti-RT treatment, suggesting that RT contributes to an epigenetic level of control. Most importantly, inhibition of RT activity *in vivo* antagonized tumor growth in animal experiments. Moreover, pretreatment with RT inhibitors attenuated the tumorigenic phenotype of prostate carcinoma cells inoculated in nude mice. Based on these data, the endogenous RT can be regarded as an epigenetic regulator of cell differentiation and proliferation and may represent a novel target in cancer therapy.
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Keywords: endogenous reverse transcriptase; proliferation; differentiation; tumor growth; RNAi; anticancer therapy

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Introduction

A striking finding emerging from the recent sequencing of the human genome is that retrotransposable elements, such as long interspersed elements (LINEs), Alu and endogenous retroviruses (ERVs) make up some 45% of human DNA (Deininger *et al.*, 2003). All classes of retroelements, but the Alu family, are endowed with a reverse transcriptase (RT)-coding gene, which enables them to retrotranspose autonomously. The presence and function of retroelements in the genome has long puzzled biologists. The lack of any obvious cellular function initially suggested that these elements, and the RT-coding sequences harbored therein, were mere evolutionary remnants and inspired the concept of 'junk DNA'. More recently, the realization that retrotransposons can reshape the genome and contribute to modulation of gene expression has led to reconsider that hypothesis. Growing evidence indicate that RT-coding genes are expressed at low levels, if at all, in differentiated nonpathological tissues; in contrast, high expression is distinctive of germ cells (Kiessling *et al.*, 1989; Giordano *et al.*, 2000), embryos (Poznanski and Calarco, 1991; Packer *et al.*, 1993), embryonic tissues (Mwenda, 1993), and undifferentiated and transformed cells (Deragon *et al.*, 1990; Martin, 1991; Martin and Branciforte, 1993), suggesting that levels of RT expression are linked to the proliferative potential of the cell. In addition, RT gene activity is upregulated by a variety of stimuli acting at the genome-wide level, for example, cellular stress (Hagan *et al.*, 2003), heat shock, cycloheximide, adenovirus infection (Li and Schmid, 2001), genotoxic agents (Hagan and Rudin, 2002), and DNA base analogs (Khan *et al.*, 2001). Unscheduled activity of retrotransposons and ERVs is implicated in a variety of diseases, including cancer (Friedlander and Patarca, 1999; Ostertag and Kazazian, 2001). Conversely, inactivation of specific RT-encoding elements using antisense oliogonucleotides or ribozymes inhibited proliferation of human (Kuo *et al.*, 1998) and murine (Crone *et al.*, 1999) cell lines. The question remains unanswered as to whether retroelements are to

be regarded as endmarkers, or causative triggers, in processes associated with cell proliferation and function.

We showed previously that non-nucleosidic RT inhibitors directed against the HIV-encoded RT in fact also inhibit the endogenous RT activity present in early embryos (Pittoggi *et al*., 2003) and undifferentiated cells (Mangiacasale *et al*., 2003). These RT inhibitors displayed powerful effects in nondifferentiated, or dedifferentiated cells: they inhibited cleavage in murine early embryos, yielding developmental arrest before the blastocyst stage (Pittoggi *et al*., 2003), and, moreover, reduced proliferation and facilitated the onset of differentiation in murine and human cell lines (Mangiacasale *et al*., 2003). These data suggest that the endogenous cellular RT plays a physiological role in cell proliferation and differentiation. Furthermore, there appears to be a requirement for regulated RT activity in differentiated cells. Here we have taken a step further and have asked whether the endogenous RT plays a direct role in proliferation and differentiation of transformed cells, and, if so, whether modulating RT activity in cancer cells might represent a novel approach to inhibit tumor growth.

Results

RT inhibitors reversibly reduce cell proliferation in human transformed cell lines

In previous work, we reported that the RT inhibitor nevirapine, widely used in anti-HIV therapy, blocks the enzymatic activity of endogenous RTs in noninfected proliferating cells, as revealed using a highly sensitive RT–PCR based *in vitro* assay (Pyra *et al*., 1994), and concomitantly reduces the rate of proliferation (Mangiacasale *et al*., 2003). Here we set out to investigate the response of human transformed cell lines to prolonged exposure to RT inhibitors. Two well-characterized RT inhibitors, that is, nevirapine and efavirenz, were used. Cells from A-375 melanoma, PC3 prostate carcinoma and TVM-A12 primary melanoma-derived lines were passaged, counted and replated every 96 h with continuous drug readdition (or DMSO alone in control cultures) for at least five 96 h-cycles. As shown in Figure 1a, both inhibitors effectively reduce cell growth in all cell lines, with a stable effect during prolonged exposure. Growth inhibition was reversible: when RT inhibitors were removed, proliferation was resumed at a comparable rate to controls within one or two 96 h-cycles. Readdition of the drugs inhibited again proliferation in all cell lines. Thus, the reduction of cell growth associated with RT inhibition is not inherited as a permanent change through cell division.

We next asked whether either RT inhibitor induced cell death in A-375 or PC3 cell lines. Combined staining with PI to reveal permeable necrotic cells, DAPI to visualize apoptotic nuclei, and DiOC6(3) to monitor the loss of mitochondrial transmembrane potential, revealed no significant induction of cell death by either RT inhibitor; what low ratio was recorded (15% at most after 72 h of exposure to either drug) was largely accounted for by apoptosis (data not shown). Thus, neither drug has significant nonspecific toxicity. We next

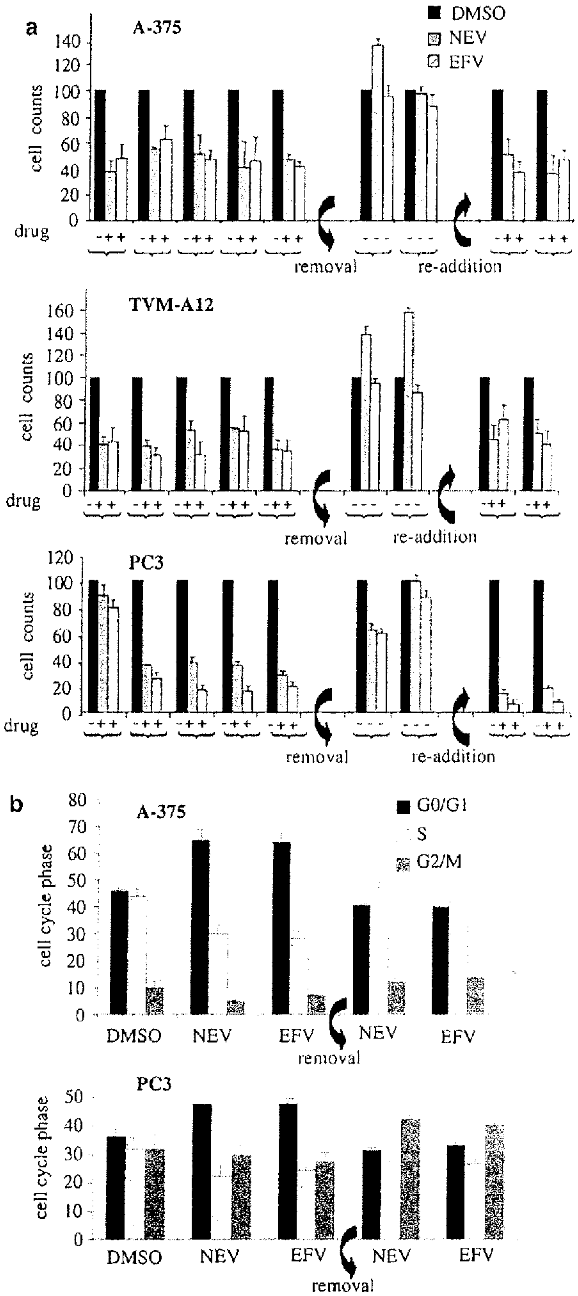

Figure 1 Inhibition of proliferation by anti-RT drugs. (**a**) Cell growth in cultures treated with DMSO (control), nevirapine (NEV) and efavirenz (EFV). Cells were counted and re-plated every 96 h for five cycles. Cells were then cultured in inhibitor-free medium (two cycles). RT inhibitors were then re-added for two cycles. Counted cells are expressed as the % of controls, taken as 100. Values represent pooled data from three experiments. (**b**) Cell cycle profiles in the presence of RT inhibitors for four 96 h-cycles and after drug removal

sought to establish whether reduced cell growth rather reflected the induction of cell cycle delay. Biparametric FACS analysis was employed to measure the DNA content (revealed by PI) and DNA replication (by BrdU incorporation) after four 96 h-cycles of exposure to RT inhibitors. This depicted significantly altered cell cycle profile in anti-RT treated cultures, with an increased proportion of G0/G1 BrdU-negative cells, that was especially pronounced in A-375 cell cultures (Figure 1b). Removal of the drugs re-established the original cell cycle profile and abolished the G1 delay.

Nevirapine induces morphological differentiation and modulates gene expression in transformed cell lines

Melanomas are resistant to most therapeutic treatments: thus, it was relevant to determine whether RT inhibitors induced differentiation concomitant with reduced cell growth. We first examined A-375 melanoma cells, which acquire a typical dendritic-like phenotype in response to certain inducers of differentiation (Sauane *et al*., 2003). As shown in Figure 2A, morphological differentiation, revealed by cell shape, dendritic-like extensions and increased adhesion, became evident within 4–5 days of exposure to nevirapine (d) or efavirenz (g), compared to DMSO-treated controls (a). By scanning electron microscopy (SEM), A-375 cells cultured with nevirapine (e) and efavirenz (h) become flattened compared to untreated controls (b) and exhibit elongated dendrite extensions that adhere tightly to the substrate. Confocal microscopy after α-tubulin immunofluorescence also revealed the reorganization of microtubule arrays throughout the length of outgrowing dendrites in RT-inhibited cells (f, i), different from controls (c), in which short microtubules concentrate around the nucleating centers. Nevirapine treatment induced similar changes in TVM-A12 primary cells derived from melanoma (Figure 2B): untreated cells have a spindle-shaped morphology by phase contrast (a) and SEM (b); nevirapine-treated TVM-A12 cells formed instead typical branched dendrites (d, e) and displayed well-organized, elongated microtubule arrays (f), compared to untreated cells (c). Significant morphological changes were also induced in PC3 prostate carcinoma cells upon exposure to nevirapine (Figure 3c and d) and efavirenz (e, f) compared to controls (a, b). The microtubule network was reorganized, with the appearance of fusiform extensions protruding from the cell periphery, particularly in response to nevirapine.

The induction of morphological differentiation suggests that critical regulatory genes are modulated in response to the RT inhibitory treatment. This was investigated in semiquantitative RT–PCR analysis of

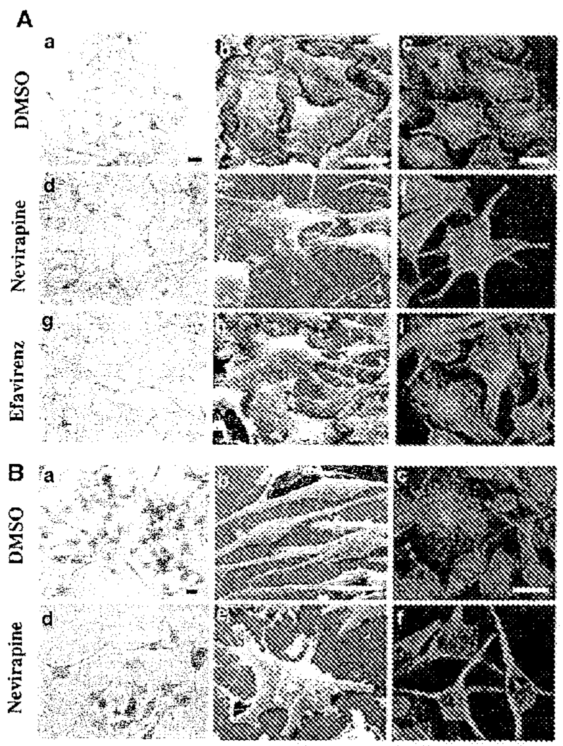

Figure 2 Morphological differentiation of melanoma cells in the presence of RT inhibitors. (A) A-375 cell line cultured in DMSO- (a–c), nevirapine- (d–f) or efavirenz- (g–i). Cultures were observed by phase-contrast microscopy after Wright Giemsa staining (left column), SEM (middle column) and confocal microscopy (right column) after α-tubulin (*green*) and PI staining of nuclei (*red*). (B) Primary melanoma-derived TVM-A12 cells. DMSO- (a–c) and nevirapine-treated (d–f) cells under phase contrast (left column), SEM (middle column), and confocal microscopy (right column). Bar, 20 μm

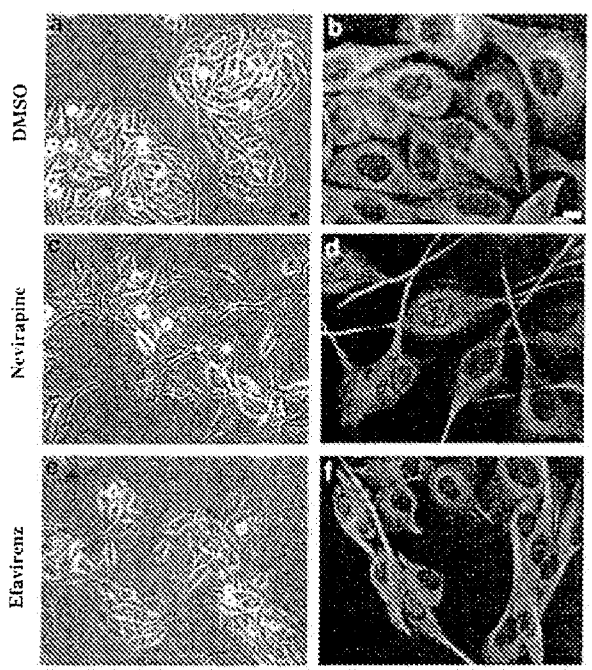

Figure 3 Morphological differentiation of PC3 prostate carcinoma cells by RT inhibitors. DMSO- (**a**, **b**), nevirapine- (**c**, **d**) and efavirenz- (**e**, **f**) treated PC3 cells under phase-contrast microscopy (**a**, **c**, **e**), and fluorescence microscopy (**b**, **d**, **f**) after α-tubulin (green) and DAPI staining of nuclei (blue). Bars, 10 μm

cultures treated with DMSO only, or nevirapine or efavirenz for four cycles. In A-375 melanoma cells, we focussed on a set of four genes: the *E-cadherin* gene, involved in cell–cell adhesion and expressed in differentiated but not in tumor cells (Hsu *et al*., 2000); and the *c-myc*, *bcl-2* (Utikal *et al*., 2002) and *cyclin D1* (Sauter *et al*., 2002) genes, which are directly implicated in melanoma cell proliferation and tumor growth. Results in Figure 4a indicate that the *E-cadherin* gene is markedly upregulated, whereas *c-myc*, *bcl-2* and *ccnd1* genes are downregulated, in RT-inhibited A-375 cultures compared to controls. One exception was recorded for efavirenz, which failed to downregulate *ccnd1* expression. We also analysed PC3 prostate carcinoma cells and selected two marker genes of differentiated prostate epithelia, that is, the prostate-specific antigen *PS-A* (Lilja, 2003) and androgen receptor (*AR*) (Linja *et al*., 2001) genes. Neither of these genes is expressed in untreated cultures, yet both genes were induced in response to RT inhibitors (Figure 4b). Again, the expression of all genes returned to the original level when the inhibitors were removed. Thus, RT inhibitory drugs modulate the expression of critical genes in transformed cells, consistent with the induction of differentiation, yet this reprogramming is reversible and is abolished when RT-inhibition is released.

RNA interference (RNAi) against RT-encoding LINE-1 elements reduces proliferation and promotes differentiation in melanoma cells

At this point, we wanted to ascertain unambiguously whether reduction of cell growth and induction of differentiation by pharmaceutical RT inhibitors are specifically attributable to the inhibition of the cellular RT. To address this question, RNAi experiments were designed to target specifically LINE-1 element families that are most abundantly expressed in human cells (Brouha *et al*., 2003, and Supplementary information). Double-stranded RNA oligonucleotides directed against LINE-1 ORF1 (L1-i, Figure 5a), or the lamin A/C gene (lam), or representing a noninterfering sequence (n.i.),

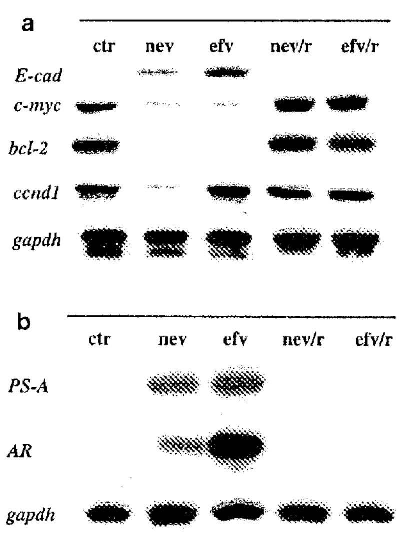

Figure 4 RT inhibitors modulate gene expression in A-375 (**a**) and PC3 (**b**) cell lines. RNA extracted from cells treated with DMSO (**ctr**), nevirapine (**nev**) or efavirenz (**efv**), and after removal of nevirapine (**nev/r**) or efavirenz (**efv/r**), was amplified by RT–PCR, blotted and hybridized with internal oligonucleotides

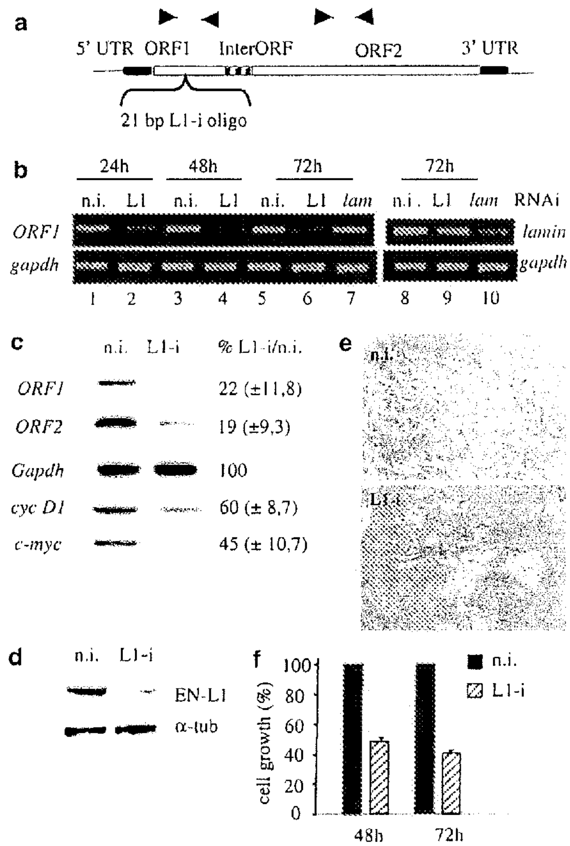

Figure 5 RNAi to LINE-1 induces morphological differentiation, reduces proliferation and modulates gene expression in A-375 cells. (**a**) Structure of a full-length LINE-1 element. The position of the siRNA oligonucleotide L1-i is indicated. Arrowheads indicate the positions of primer pairs used for RT–PCR analysis. (**b**) RT–PCR analysis of LINE-1-ORF1 (upper panel) and GAPDH (lower panel) in RNA extracted from cells 24, 48 and 72 h after transfection of L1-i (lanes 2, 4 and 6) or noninterfering (n.i., lanes 1, 3 and 5), or siRNA to the lamin A/C gene (lam, lane 7). Lanes 8, 9 and 10 show RT–PCR reactions of lamin (upper panel) or GAPDH (lower panel) using RNA from cells transfected with noninterfering, LINE-1 and lamin siRNAs, respectively. (**c**). Gene expression patterns after semiquantitative RT–PCR in A-375 cells 72 h after transfection with noninterfering or L1-i oligonucleotides. Quantitative variations (expressed as the % of signal in L1-i to signal in n.i.-transfected cultures) represent the mean and s.d. from three independent experiments. (**d**) Immunoprecipitated RT protein from L1-i and n.i.-transfected cultures using L1-EN antibody. An aliquot of total extract was analysed by Western blot using α-tubulin to control equal protein input in the immunoprecipitation. (**e**) Phase-contrast microscopy of A-375 cultures transfected with n.i. and L1-i oligonucleotides for 72 h. (**f**) Cell growth after transfection with n.i. and L1-i oligonucleotides. Counted cells are expressed as the % of n.i. controls, taken as 100

were transfected in A-375 cells. Semiquantitative RT–PCR analysis 24, 48 and 72 h after transfection indicated that LINE-1 expression is progressively reduced in LINE-1-interfered cultures (Figure 5b, lanes 2, 4 and 6) compared to controls treated with noninterfering (lanes 1, 3 and 5), or lamin RNAi (lane 7) in which lamin expression is abolished (lane 10). Conversely, lamin gene expression was not affected in LINE-1-interfered cells (lane 9). Neither LINE-1 nor lamin RNAi influenced GAPDH expression. Thus, our RNAi conditions target specifically LINE-1 expression. At 72 h after L1-i transfection, expression of both ORF1 and ORF2 was reduced by almost 80% compared to cells transfected with noninterfering oligonucleotide (Figure 5c). To ascertain that the RT protein product was consistently downregulated in L1-i interfered cells, we made use of a recently developed antibody against LINE-1 ORF2-encoded RT (termed EN-L1, Ergun *et al.*, 2004). Immunoprecipitation and Western blot assays indicate that RT protein levels 72 h after transfection are significantly reduced in L1-i cells (Figure 5d). Remarkably, L1-i interfered cultures, in which the RT protein was downregulated, developed a typical differentiated morphology (Figure 5e), concomitant with reduced cell growth (Figure 5f), compared to cells transfected with noninterfering oligonucleotide. Furthermore, LINE-1 interference induced downregulation of expression of *c-myc* and *cyclin D1* genes, but not of *GAPDH* (Figure 5c). These effects are comparable to those obtained with pharmacological RT inhibition.

RT inhibitors reduce the growth of human tumor xenografts in athymic nude mice

Given that proliferation and differentiation can be modulated by RT inhibition in transformed cells, we next asked whether RT inhibitors also affect tumor growth *in vivo*. Tumorigenic cell lines selected for these experiments include A-375 and PC3 lines, as well as HT29 colon and H69 small cell lung carcinoma lines, which also showed reduced cell growth in response to RT inhibitors (Mangiacasale *et al.*, 2003, and data not shown). Cells were inoculated subcutaneously in the limb of athymic nude mice. Animals were then subjected to treatment with efavirenz, because this drug showed a higher *in vivo* effectiveness than nevirapine in preliminary assays. The optimal dose (20 mg/kg body weight) was determined in dose-response experiments testing 4–40 mg/kg of the drug. The efavirenz treatment proved safe in all animal groups, with no animal death or explicit signs of toxicity in any of the groups – although the group treated with 40 mg/kg showed a significant decrease of body weight in more than 60% of animals. Figure 6 shows the recorded curves of tumor growth in mice untreated (red) or treated with efavirenz, starting one day (purple), or 1 week (yellow), after tumor inoculation. Tumor growth was markedly reduced in treated compared to untreated animals for all xenograft types, and tumor progression was antagonized with comparable effectiveness regardless of the timing of the treatment start, despite of differences in the initial tumor size. The growth curves of PC3- and HT29-derived tumors in animals treated from day one after inoculation, but subjected to treatment discontinuation after day 15 (green curves), indicate that the inhibition of tumor growth requires continuous RT inhibition *in vivo*.

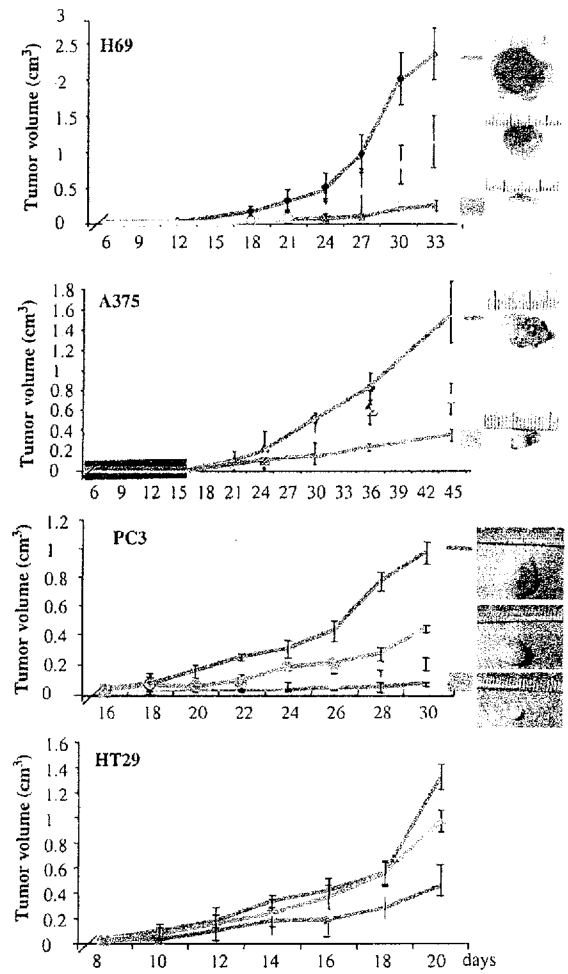

Figure 6 Efavirenz treatment reduces human tumor growth in nude mice. The growth of tumors formed by the indicated cell lines was monitored in untreated animals (red) and in animals treated with efavirenz 1 day (purple) or 1 week (yellow) after inoculation. Green curves show the growth of PC3- and H69-derived tumors in animals treated starting 1 day after inoculation and subjected to treatment discontinuation after 14 days. Curves show the mean value of tumor size in groups of five animals

Efavirenz-treated PC3 cells exhibit reduced tumorigenicity in vivo

Finally, we asked whether pretreatment of transformed cells with efavirenz modifies the tumorigenic potential of derived xenografts. PC3 prostate cancer cells were cultured with efavirenz for two 96 h cycles, a time that was sufficient for induction of the *PS-A* and *AR* genes in cultured cells (Figure 4b), and subsequently inoculated

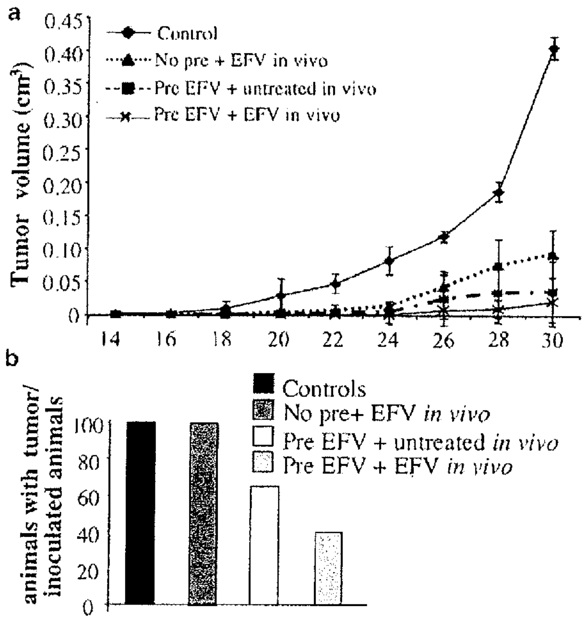

Figure 7 Reduced tumorigenicity of PC3 cells pretreated with efavirenz. (**a**) Growth of tumors formed by untreated or efavirenz pretreated cells injected in mice that were not treated or were post-treated with efavirenz *in vivo*. (**b**) The outcome of PC3-derived xenografts after the indicated treatments for 30 days ($n = 20$ animals/group)

in nude mice. Untreated cells were inoculated in parallel batches of animals. Efavirenz-pretreated, or untreated, PC3 cell xenografts were then either continuously treated with efavirenz *in vivo* or were left untreated. Figure 7a shows the rate of tumor growth in these experiments: untreated PC3 cells developed fast-growing tumors in all animals. In contrast, efavirenz-pretreated PC3 cells showed a significantly reduced tumor-forming ability *in vivo*, and xenografts grew more slowly. Figure 7b shows the incidence of tumors in animals models exposed to different treatments: while 100% of inoculated animals developed aggressive tumors using untreated PC3 cells, efavirenz-pretreated cells developed slowly-growing xenografts in 65% of the inoculated animals. Moreover, only 40% of the animals inoculated with pretreated cells and further treated with efavirenz *in vivo* developed a tumor at all, and in that case the growth curve was flat. Thus, pretreatment of cells with anti-RT drugs before inoculation attenuates the tumorigenic potential of transformed cells.

Discussion

This work highlights three unexpected aspects of the human genome that have implications for cancer: first, LINE-1 elements are identified as active components of a mechanism involved in control of cell differentiation and proliferation; second, RNAi-dependent inactivation of LINE-1 elements, or pharmacological inhibition of the endogenous RT activity which they encode, can restore control of these traits in transformed cells; third, inhibitors of RT reduce tumor growth in animal models *in vivo*.

The RT inhibitors used here, nevirapine and efavirenz, share a common biochemical mechanism of action by binding the hydrophobic pocket in the p66 subunit of RT enzymes (Di Marzo Veronese *et al*., 1986; Ren *et al*., 2001). Though being designed to target the HIV-encoded RT, nevirapine proved able to inhibit the endogenous retrotranscriptase activity present in non-infected cells (Mangiacasale *et al*., 2003) in a highly sensitive *in vitro* assay (Pyra *et al*., 1994). We now show that both drugs reduce proliferation of transformed cells, largely independent of cell death, but associated with G1 delay or arrest. Concomitant with this, RT inhibitors induce morphological differentiation of transformed cells. The induction of differentiation is rapid, different from phenotypic changes elicited by inhibitors of the telomerase-associated RT (TERT), which require long treatment times (120 days) (Damm *et al*., 2001). Furthermore, we never observed the reorganization of actin stress fibers or focal adhesion sites typical of senescent cells. The absence of senescence-specific modifications, and the rapid induction of differentiation, indicate that the RT inhibitors do not target TERT and induce a low-proliferating differentiated phenotype rather than senescence.

That these effects are specifically associated with RT inhibition was further demonstrated in RNAi experiments targeting active LINE-1 retroposon families accounting for 84% of the overall retrotransposition capability in human cells (Brouha *et al*., 2003). In A-375 cells, our RNAi conditions specifically downregulate expression of LINE-1 ORF1 and ORF2 by some 80%, suggesting that the biologically active LINE-1 subgroup was effectively silenced. Consistently, RT protein levels were also downregulated in LINE-1-interfered cells. RNAi to RT encoding LINE-1 elements induced morphological, proliferative and gene-profiling changes that are virtually indistinguishable from those caused by pharmacological RT inhibitors. The similarity of phenotypes induced by independent approaches indicates that inhibition of LINE-1 expression, or of RT activity, is sufficient to delay proliferation and promote differentiation. These observations rule out that non-specific side effects of the drugs cause the observed cell phenotypes and highlight the specificity of the role of RT.

Consistent with growth reduction and induction of differentiation, RT inhibition caused the reprogramming of gene expression: this implicates the endogenous RT in modulation of expression of genes that promote the transition from highly proliferating, transformed phenotypes to low proliferating, differentiated phenotypes, suggesting that genome function is the ultimate target of pharmaceutical or RNAi-dependent inhibition of RT activity. Changes in gene expression are not inherited through cell division, but are reversible when RT inhibition is released. The reversibility of examined features after release of the inhibition suggest that LINE-1 encoded RT is part of an epigenetic mechanism

that can modulate gene expression and contributes to the molecular mechanisms underlying cell proliferation and differentiation.

A relevant finding in this study is the ability of RT inhibitors to reduce tumor growth in nude mice inoculated with four human xenograft models *in vivo*. Tumor growth was inhibited as long as the animals were supplied with RT inhibitor, yet was resumed on discontinuation of the treatment, as observed in cell lines, consistent with an epigenetic role of the endogenous RT in tumor growth. These data illustrate the promising cytostatic ability of RT inhibitors in cancer treatment. Furthermore, *in vitro* pretreatment of PC3 prostate carcinoma cells with efavirenz attenuates their tumorigenicity *in vivo*. Thus, the activation of differentiation markers and reduced proliferation associated with RT inhibition are part of a large-scale reprogramming that can attenuate the malignant phenotype of transformed cells *in vivo*.

Growing data indicate that epigenetic changes can reprogram tumor cells and convert the transformed phenotype into a 'normal' non pathological state (Lotem and Sachs, 2002; Li *et al*., 2003). Epigenetic reprogramming can bypass the genetic alterations that originally caused the malignant transformation in a variety of tumors (Lotem and Sachs, 2002). Therefore, epigenetic regulatory factors are viewed as valuable, worth-challenging targets in tumor therapy (Egger *et al*., 2004). Retrotransposons can contribute to heterochromatin formation in fission yeast (Schramke and Allshire, 2003). Though such a mechanism has not yet been proved in higher eukaryotes, unpublished results in our laboratory suggest that LINE-1-encoded RT is implicated in nuclear reorganization and positioning of specific genes. Importantly in this respect, the intranuclear position of genes is directly linked to their expression (Osborne *et al*., 2004 and references herein).

Of relevance to the present study is the observation that – while many tested compounds targeting the 'epigenome' have generally proven toxic and/or chemically unstable – both nevirapine and efavirenz have been used in AIDS treatment for many years. Thus, the prospect of using these RT inhibitors in cancer therapy would have obvious advantages given their epidemiological record of generally good tolerance to continued administration. In retrospect, epidemiological evidence indicate that Kaposi's sarcoma (Portsmouth *et al*., 2003) and other AIDS-related cancers (Tirelli and Bernardi, 2001) have a reduced incidence in patients treated with highly active antiretroviral therapy (HAART): while this is generally viewed as a reflection of the improved immune reaction in treated patients, it may also suggest a direct inhibitory effect of HAART on the endogenous RT activity in tumor cells.

Materials and methods

Cell cultures

Human A-375 melanoma (ATCC-CRL-1619), TVM-A12 primary melanoma-derived (Melino *et al*., 1993), HT29 adenocarcinoma (ATCC HTB-38), H69 small-cell-lung carcinoma (SCLC) (ATCC HTB119), and PC3 prostate carcinoma (ATCC CRL-1435) cell lines were seeded in six-well plates at a density of 10^4 to 5×10^4 cells/well and cultured in DMEM or RPMI 1640 medium with 10% fetal bovine serum. Nevirapine and efavirenz were purified from commercially available Viramune (Boehringer-Ingelheim) and Sustiva (Bristol-Myers Squibb) as described (Pittoggi *et al*., 2003). The drugs were made 350 and 15 μM in dimethyl sulfoxide (DMSO, Sigma-Aldrich), respectively, and added to cells 5 h after seeding; the same DMSO volume (0.2% final concentration) was added to controls. Fresh RT inhibitor-containing medium was changed every 48 h. Cells were harvested every 96 h, counted in a Burker chamber (two countings/sample) and replated at the same density.

Cell cycle and cell death analysis

BrdU (20 μM) was added to the cultures during the last 30 min before harvesting. Harvested cells were then treated with anti-BrdU antibody and propidium iodide (PI) and subjected to biparametric analysis of the DNA content and BrdU incorporation in a FACStar Plus flow-cytometer (Beckton-Dickinson). Cell death was assessed by microscopy after combined staining with DAPI (nuclear morphology); PI (cell permeability); and 3,3 dihexyl-oxacarbocyanine [DiOC6(3)], a fluorescent probe for mitochondrial transmembrane potential.

Indirect immunofluorescence and confocal microscopy

Cell preparations were fixed with 4% para-formaldehyde for 10 min and permeabilized in 0.2% Triton-X100 in PBS for 5 min. Mouse monoclonal anti-bovine α-tubulin (Molecular Probes, A-11126) was revealed by Alexa Fluor 488-conjugated secondary antibody (Molecular Probes, A-11001) in A-375 and TVM-A12 cells and FITC-conjugated secondary antibody (Jackson Immunoresearch, cat 115-095-068) in PC3 cells. Nuclei were stained either with 2 μg/ml PI in the presence of 0.1 mg/ml ribonuclease A or with 0.1 μg/ml DAPI. Samples were imaged under a confocal Leica TCS 4D microscope equipped with an argon/kripton laser. Confocal sections were taken at 0.5–1 μm intervals.

Scanning electron microscopy (SEM)

Cells were fixed in 2.5%. glutharaldehyde in 0.1 M Millonig's phosphate buffer (MPB). After washing, cells were postfixed with 1% OsO_4 (1 h, 4°C) in MPB and dehydrated using increasing acetone concentrations. Samples were critical-point dried using liquid CO_2 and sputter-coated with gold before examination on a Stereoscan 240 scanning electron microscope (Cambridge Instr., Cambridge, UK).

Semiquantitative RT–PCR

RNA extraction and treatment with RNase-free DNase I were as described (Pittoggi *et al*., 2003). cDNAs were synthesized using 300 ng of RNA, oligo (dT) and the Thermoscript system (Invitrogen). Reaction mixtures (1/25) were amplified using the Platinum *Taq* DNA Polymerase kit (Invitrogen) and 30 pmol of oligonucleotides (MWGBiotech, Ebersberg, Germany; see Supplementary information) in an initial 2-min step at 94°C, followed by cycles of 30 s at 94°C, 30 s at 58–62°C, 1 min at 72°C. Each oligo pair was used in sequential amplification series with increasing numbers (25–40) of cycles. PCR products were electrophoresed, transferred to membranes and hybridized for 16 h at 42°C with [^{32}P]γ-ATP end-labeled internal oligonucleotides. The intensity of the amplification signal was

measured by densitometry in at least three independent experiments for each gene and normalized to the GAPDH signal in the same experiment.

RNA interference

Four 21-nt double-stranded siRNA oligonucleotides encompassing region 1367–2056 in LINE-1 were designed to target the consensus sequence of active LINE-1 elements described by Brouha *et al.* (2003). The siRNA oligonucleotide targeting bases 2035–2056 (L1-i) was most effective and was used in all experiments. Control cells were treated with noninterfering oligonucleotide (n.i.), 3′-fluorescein-conjugated to monitor transfection efficiency, or with specific siRNA against the lamin A/C gene. All siRNAs were synthesized by Qiagen USA. A-375 melanoma cells were transfected using RNAiFect transfection reagent (Qiagen) and 300 nM of siRNA. Details for siRNA assays are in Supplementary information.

Western blot and immunoprecipitation

At 72 h after siRNA transfection A-374 cells were harvested in PBS with 0.1 mM. PMSF and lysed in lysis buffer (50 mM Tris-HCl, pH 8.1, 10 mM EDTA, 1% SDS) supplemented with protease inhibitors (1 μM pepstatin, 1 μM leupeptin, 0.1 mM PMSF). After centrifugation at 12 000 r.p.m., 4°C, 15 min, the protein concentration in the supernatants was determined using a Coomassie colorimetric assay (Pierce). Samples (20 μg) were loaded on NuPAGE Novex 10% Bis-Tris gel (Invitrogen), transferred onto membranes and verified by Western immunoblotting using monoclonal anti-α-tubulin (Sigma, T5168) and HRP-conjugated secondary antibody (BIORAD, 170-6516). In total, 500 μg of protein extract were then precleared using 75 μl of protein A-Agarose-50% Slurry beads (Upstate Biotechnology) for 30 min at 4°C. After centrifugation (12 000 r.p.m., 4°C), supernatants were incubated overnight (4°C, with continuous rotation) with chicken polyclonal anti-EN-L1 (Ergun *et al.*, 2004), kindly given by Gerald Schumann (Paul-Ehrlich-Institut, Langen, Germany). Precleared extracts were then incubated with 60 μl of beads (1 h, 4°C) with rotation. After removal of the supernatants, proteins were eluted from the beads in 1% SDS, 0.1 M $NaHCO_3$ and precipitated with 10 volumes of acetone. Pellets were resuspended in TE buffer and loaded on NuPAGE Novex 10% Bis-Tris gel as above. Western blot analysis was carried out using chicken polyclonal anti-EN-L1 (1 : 40 dilution) and donkey HRP-conjugated anti-chicken IgY (Jackson Immunoresearch Laboratories, 703-035-155).

Tumor xenografts and treatment of animals

Athymic nude mice (5 weeks old) (Harlan, Italy), kept in accordance with the European Union guidelines, were inoculated subcutaneously with A-375 melanoma (4×10^6), H-69 (10^7), PC3 (2×10^6) and HT-29 (10^6) cells in 100 μl PBS. Mice were subcutaneously injected daily five days a week with Efavirenz (20 mg/kg) using a 4 mg/ml stock in DMSO freshly diluted 1 : 1 with physiological solution. Controls were injected with 50% DMSO. Treatment started 1 day or 1 week after tumor implant, and, where indicated, was discontinued after 14 days. Tumor growth was monitored every other day by caliper measurements; volumes were calculated using the formula length × width × height × 0.52 (Umekita *et al.*, 1996).

Acknowledgements

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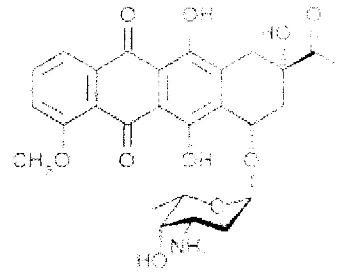
Supplementary Information accompanies the paper on Oncogene website (http://www.nature.com/onc)

Exhibit C: Anthracyclin definition from www.wikipedia.org

Anthracycline

From Wikipedia, the free encyclopedia

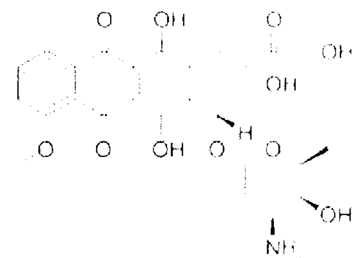
Anthracyclines (or **anthracycline antibiotics**) are a class of drugs used in cancer chemotherapy derived from Streptomyces bacteria [1] (more specifically, Streptomyces peucetius var. caesius).[2]



Daunorubicin, the prototypical anthracycline

These compounds are used to treat a wide range of cancers, including leukemias, lymphomas, and breast, uterine, ovarian, and lung cancers.

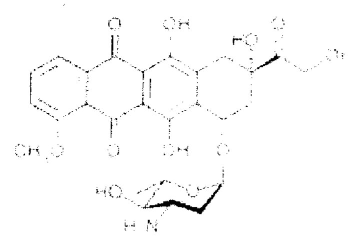
The anthracyclines are some of the most effective anticancer treatments ever developed and are effective against more types of cancer than any other class of chemotherapy agents.[3][4][5] Their main adverse effects are heart damage (cardiotoxicity), which considerably limits their usefulness, and vomiting.



Doxorubicin

The first anthracycline discovered was daunorubicin (trade name Daunomycin), which is produced naturally by *Streptomyces peucetius*, a species of actinobacteria. Doxorubicin (Adriamycin) was developed shortly after, and many other related compounds have followed, although few are in clinical use.[3]

Contents

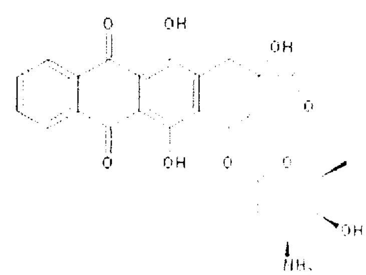


Epirubicin

Examples

Available agents include:

- Daunorubicin (Daunomycin)
- Daunorubicin (liposomal)
- Doxorubicin (Adriamycin)
- Doxorubicin (liposomal)
- Epirubicin
- Idarubicin
- Valrubicin, used only to treat bladder cancer



Idarubicin

Since they are antibiotics, anthracyclines can kill or inhibit the growth of bacteria, but because they are so toxic to humans, they are never used to treat infections.

Mechanism of action

Anthracycline has three mechanisms of action:

1. Inhibits DNA and RNA synthesis by intercalating between base pairs of the DNA/RNA strand,